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Acoustic adaptation to high and low frequency noise in the Serin (*Serinus serinus*)

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Contents

Abstract

Song is one of the most common signals used to establish relationships between birds. Mostly produced by males, songs are used to repel other males and attract females, being extremely relevant in the social behaviour and evolution of songbirds.

Regardless of the several advantages of the acoustic signals, they suffer attenuation and degradation by the surrounding environment, on the pathway from sender to receiver. Adding to that, there is sometimes a considerable amount of noise, i.e., an unwanted external interfering sound(s) that force birds to cope with masking. Abiotic factors such rain and temperature, and biotic factors as other species' vocalizations and anthropogenic noise have great impact on birdsong, affecting the receiver's perception and interpretation of the message. The plasticity of the signal to overcome these constraints dictates whether it is selected over evolutionary time.

It is now known that birds perform a series of adjustments to cope with noise masking. Those adaptations can be divided in long-term, where modifications of the signal characteristics and the use of multicomponent signals, that might have driven birdsong to change over time; and in short-term adjustments, which aggregate changes of signal characteristics (amplitude, duration, redundancy and frequencies) and signal timing.

The objective of this thesis was to assess the short-term strategies performed by male serins, *Serinus serinus*, when exposed to two types of digitally created noise: low and high-frequency stimulus. Despite its very complex song being also very rigid, changes were expected mostly to be temporal: with modifications in duration of songs and intervals between songs; and structural changes such as swifts in the number of trills and peak frequencies.

The subjects of this experiment showed temporal and structural changes. More specifically, during the three phases of the experiment, males that were exposed to low-frequency noise significantly decrease the duration and peak frequency of selected sections of their songs. No other analysed parameter has suffered any temporal or structural change with noise masking. Although individuals did not perform many adjustments, those performed show some capacity of this species to adapt and possibly overcome noise.

This work provides another evidence of adjustment to noise that might seriously affect birds' communication and consequently their population ecology.

Resumo

Canções e vocalizações são dos sinais mais comuns, usados para estabelecer relações entre aves. Produzidas maioritariamente por machos, as canções são usadas para repelir outros machos ou para atrair fêmeas, sendo extremamente relevantes no comportamento social e evoluçao dos oscinos.

Apesar das suas inúmeras vantagens, o ambiente atenua e degrada os sinais acústicos, no percurso entre o emissor e o recetor do sinal. Em adição, há por vezes uma quantidade considerável de ruído, i.e., um sinal ou sinais externos não desejados que força as aves a cooperar com o mascaramento. Fatores abióticos, como a chuva ou a temperatura, e fatores bióticos tais como as vocalizações de outras espécies e o ruído antropogénico têm grande impacto nas canções, afectando a perceção e interpretação da mensagem. A plasticidade do sinal para ultrapassar esses constrangimentos dita se este é ou não selecionado ao longo do tempo.

É sabido que as aves têm sido citadas por adotarem uma série de ajustes vocais para cooperar com o mascaramento do ruído. Essas adaptações podem ser divididas em longo-termo, onde há ajustes de características do sinal e o uso de sinais múltiplos, que poderão ter "guiado" o canto das aves ao longo do tempo; e em ajustes a curto-termo que agregam alterações de características do sinal (amplitude, duração, redundância e frequências) e ajustes temporais de canto.

O objetivo desta tese foi determinar as estratégias adotadas por machos da espécie *Serinus serinus* quando expostos a dois tipos de ruído criados digitalmente: um estímulo de baixa e outro de alta frequência. Apesar da canção desta espécie ser muito rígida e complexa, as alterações esperadas seriam maioritariamente temporais: com

modificações na duração das canções e dos intervalos entre canções; e alterações estruturais no número de trilos e nas frequências dominantes das canções.

Os sujeitos desta experiência demonstraram alterações a nível temporal e estrutural. Mais especificamente, durante as três fases da experiência, machos expostos a ruído de baixa frequência diminuíram significativamente a duração e a frequência dominante de secções das suas canções. Nenhum outro parâmetro sofreu alterações temporais ou estruturais com o mascaramento do ruído. Embora os indivíduos não tenham feito muitas modificações, as que foram efectuadas denotam uma relativa capacidade de adaptação e possível superação do ruído por parte desta espécie.

Este trabalho providencia mais uma evidência de ajuste ao ruído que poderá afetar seriamente a comunicação entre as aves e consequentemente a ecologia das suas populações.

Chapter 1   **Introduction**

1.1. Animal communication

Communication is the foundation of social relationships between animals (Greenewalt 1968; Brumm & Slabbekoorn 2005) and is defined as the act of transmitting or sharing information between different individuals, conveyed through signals (Slater 1983; Wiley 1983; Bradbury & Vehrencamp 1998; Tomecek 2009).

Communication is achieved when the intentionally emitted signal is perceived and interpreted by at least one receptor, that then decides to respond or not, and if yes, how (Slater 1983; Bradbury & Vehrencamp 1998; Rogers & Kaplan 2002; Catchpole & Slater 2008). Due to natural selection an individual should only produce a signal that benefits its fitness and the same is valid for the receptor, which should only respond to signals that benefit their fitness – true communication (Wiley 1983; Bradbury & Vehrencamp 1998). When the emitted stimuli do not benefit the sender, it is not called signal but cue (Seeley 1989 in Bradbury & Vehrencamp 1998). The benefits of a communicative interaction depend on the relation between the encoding from the producer and the decoding from the receptor. Once again, natural selection favours the maximization of the information's efficiency transmitted in a signal (Wiley 1983).

The information is conveyed through a combination of visual, chemical and acoustic signals, and also by physical contact (Slabbekoorn 2004; Tomecek 2009). Through communication animals transmit information about their identity (their species or sex), status (dominance or submission), their motivation (approach, mount or attack) or information about the surrounding habitat (presence of predator or food) (Bradbury & Vehrencamp 1998). Not only the female chooses a courting male through several signals such as song, colouration and behavioural displays, as she also uses sounds, body's shape, movements, scent or colour to let males know that she is ready to mate (Tomecek 2009).

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In birds, visual communication is very important and largely shown through displays of conspicuous plumage and colouration. Used mainly in male-male interactions and female attraction, visual signals have several disadvantages such as poor transmission over dense or dark environments that lead birds to rely also on acoustic communication. There are several characteristic that confer advantages to this type of communication: (1) capacity for long distance signalling transmission; (2) the omni-directionality, with signals being transmitted in all directions; (3) the capacity to penetrate the natural environment: signals can be used in daylight and darkness, and can penetrate and overcome obstacles such as landscape or vegetation; (4) its low energetic cost; and (5) its capacity to transmit a great amount of information in a fast and transient way (Slabbekoorn 2004; Catchpole & Slater 2008). Table I compares different characteristics of the four communication channels.

Table I. Comparison between the different communication channels.

	Acoustic	Visual	Chemical	Tactile
Nocturnal use	Good	Poor	Good	Good
Around objects	Good	Poor	Good	Poor
Range	Long	Medium	Long	Short
Rate of change	Fast	Fast	Slow	Fast
Locatability	Medium	Good	Poor	Good
Energetic cost	Low	Low	Low	Low

(Modified from Alcock 1989 in Catchpole & Slater 2008)

Birds' vocalisations can be divided in songs and calls. In taxonomic terms, the Oscines or songbirds (suborder Passeri) are the only species from the order Passeriformes with singing behaviour, possible because of the complexity of their syringeal muscles, separating them from suboscines (suborder Tyranni). Songs are longer, more complex and produced mostly by males (there are some exceptions in tropical species with duet performances), while calls tend to be shorter, simpler and

produced by both males and females, in specific situations such as flight, threat or alarm events (Catchpole 1982; Catchpole & Slater 2008).

Song has been shown to be efficient at repelling other males (establishment and territory defence), and to attract and physically stimulate females (mate attraction and stimulation) (Catchpole & Slater 2008). With that, songs' characteristics should imply the singing male quality, both to attract a mate and repel a rival. Having this dual function, the assessment by females and males must be different: females need to access the male's age, parental ability and territorial quality to maximize reproductive success; and males need to access its rival's position, willingness to attack or fighting ability to obtain resources, to access territories or even mates. In some situations, specific song characteristics may inform both males and females, but in many cases that information is very different depending on the context. The quality of the advertisement required for this dual function has driven song evolution: individuals belonging to the same species show differences in their songs, which then forces potential rivals and mates to respond accordingly to those changes (Collins 2004).

Songbirds learn their songs in early stages of life and can, in some species, continue to learn throughout the individual's life. They learn from their parents or from neighbours and repertoire size can vary enormously between species. In some, it may be constituted by several thousands of songs, while in others there are just one or a few different songs. Some species have dialects and in tropical regions many females also sing as their mates. From the top of the trees to dense forest, from relatively long songs sang for several minutes to a split second vocalizations, the diversity of birdsong is enormous (Kroodsma 2004).

Thorpe (1958) studied song learning for the first time with captively bred chaffinches, examining how their songs were affected by what they experienced. The

experiment consisted on keeping some of the young birds deprived from adult songs. The resulting songs sung by the young birds were very rudimentary: they had the right length and were sung in the same frequency, but lacked the detailed structure of wild birds' song. The other part of the study sample that had access to adult songs, sang normal song based on the ones they have heard proving the existence of a song learning process. Moreover, young birds showed a selective learning process when they didn't imitate other species songs or a tune from a whistle. With these results, it has been proved that hearing adult song is an essential requirement for the development of normal songs by young birds. Due to differences in the learning processes between different species, it is difficult to generalise, but similar results were also shown by other species (Catchpole & Slater 2008).

Learning brings many advantages to birds: it allows social and genetic adaptation, adaptation to the habitat and it is an honest indicator of male quality (Catchpole & Slater 2008). Although the learning process from the father is very important in the growing phase of a young chick, throughout time those songs are lost or written over by the acquisition of new experiences and adjustments to new environments (Kroodsma 2004). The absence of learning plasticity may, in the future, bring some negative consequences such as auditory masking by environmental noise, which in turn may affect the density and diversity of the species (Slabbekoorn & Peet 2003).

The majority of birds have repertoire of different song types of their species song. Structurally, a song is composed by syllables that occur in a particular pattern, on a simpler or more complex outline. When a song is complex, syllables are composed by elements, represented in a sonogram as a continuous line. A group of syllables is called phrase, which grouped form the distinct sections of a song (figure 1). Elements,

syllables and songs are separated by intervals that define each one of them, being the ones that separate songs the largest, and so on downwards (Catchpole & Slater 2008).
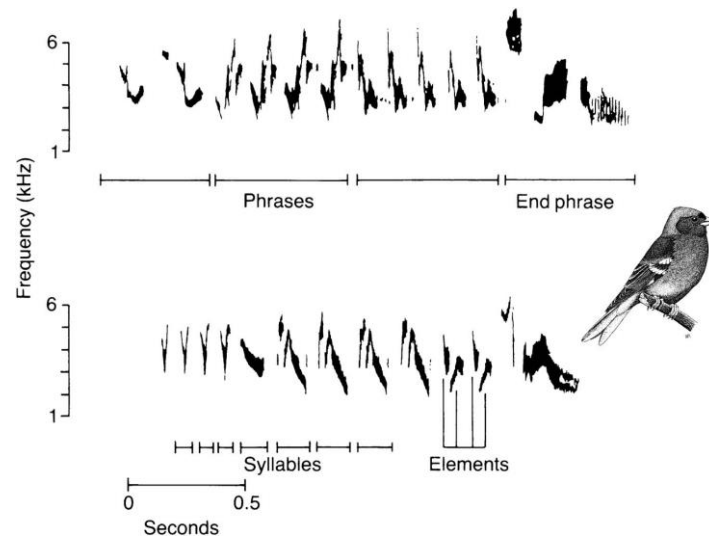
Figure 1. Sonograms of two different song types of a male chaffinch illustrating the different components of a song: phrases, syllables and elements (Slater & Ince 1979, in Catchpole & Slater 2008).

When analysing sound, there are common terms essential to its characterization, presented in the glossary (Catchpole & Slater 2008).

Glossary
Sound waves: pressure changes in the medium, which in the case of birds is always the air. It is measured in microbars.
Wavelength: length of one complete wave cycle, measured in millimetres.
Amplitude: represents the height or volume of the sound waves; measured in decibels (dB).
Frequency: number of cycles per second, indicating how high or low pitched the sound. It is measured in thousands of cycles per second or kilohertz (kHz)
Peak frequency: frequency of highest intensity (or volume).

The primary sound-generating organ of birds is the syrinx, surrounded by the interventricular air sac, at the base of the trachea (Brackenbury 1982). In songbirds, at the end of each bronchus, there are the labia formed by connective tissue and adducted into the syringeal lumen, which then vibrate producing sound (Hauser & Konishi 1999; Catchpole & Slater 2008). The complexity of birdsong might be explained by the presence of a complex of five pairs of muscles that constitute the syrinx (Catchpole & Slater 2008) and by its location, i.e., being located at the bronchial junction, the syrinx has then the capability to produce two sounds, one from each bronchus (Catchpole & Slater 2008).

On the pathway from the syrinx to the exterior, by lengthening and shortening the vocal tract, birds can adjust their acoustic signals. That can be achieved by adjusting the neck length and by opening and closing the bill – bill widely open allows the production of high frequency sounds and closed allows the production of lower frequencies. Since trilled syllables have a wide frequency range, there's the need to often open and close the bill (Catchpole & Slater 2008). However, the wider is the frequency, the lower is the maximum rate of production (Podos 1997).

The other half that makes the communication system is the capacity to efficiently detect and discriminate a sound. That part is put together by the ear along with the auditory pathway till the auditory neurons in the brain, being auditory field L the main receptive area. Changes in pressures cause the tympanic membrane to vibrate, vibrations that are transmitted by the columella to the inner ear. However, not only is important the capability to detect a sound, but also to localize it (Catchpole & Slater 2008).

1.2. Ecology of birdsong

Regardless of the many advantages or acoustic signals over visual, chemical or physical contact, there is an effect of (1) attenuation and (2) degradation of sounds by the surrounding environment (Wiley & Richards 1982; Forrest 1994). According to the flexibility of the species, there will be different ways and levels of adaptation to successfully communicate acoustically by overcoming most of the obstacles (Slabbekoorn 2004). The signal that is plastic enough to overcome them is selected over evolutionary time, being with that a form of adaptation of an animal to its environment (Bradbury & Vehrencamp 1998).

Along the transmission of an acoustic signal there is a rapid loss of energy along the spherical spreading of the sound, resulting in attenuation at 6 dB per doubling distance between sender and receiver – spherical spread of sound energy (Wiley & Richards 1982; Slabbekoorn 2004). Attenuation is then the progressive reduction of sound intensity or distortion with distance (Wiley & Richards 1982; Catchpole & Slater 2008). Taking in account that birds do not sing in homogeneous habitats, attenuation tends to be greater in natural conditions – excess attenuation –, which varies with frequency, weather and habitat conditions (Wiley & Richards 1982; Catchpole & Slater 2008). With an increase of frequency, there is an increase of the absorption and scattering of sound energy by the atmosphere (Wiley & Richards 1982) and high frequency sounds tend to be more easily attenuated by absorption in hot and humid habitat and scattered by bouncing obstacles. Low frequency sounds, on the other side, have longer wavelengths which allow them to be more resistant to objects and atmospheric changes (Catchpole & Slater 2008).

Song not only gets quieter (or attenuated) but it is also distorted and degraded with distance (Catchpole & Slater 2008). Degradation denotes changes in spectral, temporal and structural characteristics in the transmission pathway of the sound. The ratio between the wavelength of the signal and the size of the obstacle determines how much a sound is reflected: the higher the frequency of the sound, the more a small obstacle affects it. Thus, low frequency signals are more easily transmitted through vegetation and barely affected, when in comparison with high frequency ones (Wiley 1983; Slabbekoorn 2004). Degradation by reverberation is particularly problematic in habitats with dense vegetation that imply reflections and echoes, provoking the slurring of acoustic elements (Wiley & Richards 1982; Catchpole & Slater 2008). Another degradation process is caused by atmospheric turbulence, more intense in open field sites, that provokes irregular amplitude (or volume) changes of the signal. For both processes, higher frequency sounds are more easily affected and degradation is higher with the increase of the distance between sender and receiver (Wiley & Richards 1982).

1.3. Communication in noisy environments

Communication is affected by environment's filtering, absorption by the medium and noise (Forrest 1994). The term noise or background noise is used to describe any unwanted external interfering sound(s) in the background during the transmission of an acoustic signal. It is ubiquitous and many times quite considerable (Brumm & Slabbekoorn 2005). Background noise can be continuous or discrete (e.g. waterfall vs. thunder, busy highway vs. small road), can have broad or narrow frequency band, and can be predictable (e.g. bird choruses at dawn) or not (Brumm & Slabbekoorn 2005). The main source of background low-frequency noise is the wind

passing over vegetation and even the receiver's body itself (Brumm & Slabbekoorn 2005). Other abiotic factors such as rain, temperature and humidity also attenuate the amplitude of the acoustic signal, leading to a distortion in perception. Adding to all those obstacles, there is also the presence of biotic factors, such as the vocalizations from other species (Forrest 1994; Slabbekoorn 2004; Brumm & Slabbekoorn 2005). In some places, other bird chorus are the main source of biotic noise (Catchpole & Slater 2008) but also with the increase and expansion of the Human population, there is a consequent increase of anthropogenic noise and the invasion of many animals' territories, challenging birds to overcome more obstacles (Brumm & Slabbekoorn 2005).

On noise research it has been assumed that the recipient compares the input in two different ways: (1) with only the background noise and (2) with a signal added to the existing noise. Without the knowledge of the exact time that a signal will be emitted, the receiver must be doing a continuous auditory scan to select signals of interest (Klump 1996).

Masking caused by background noise reduces signal-to-noise ratios which might cause problems on the reception and interpretation of the information (Klump 1996). That ratio represents the degree to which signals are able to stand out against noise, and so signals are more easily detected and discriminated with higher signal-to-noise ratios (Forrest 1994; Klump 1996; Brumm & Slabbekoorn 2005). Noise profiles found in cities are mainly low frequency, with most energy under 2 kHz, so signals with frequencies over 2 kHz, would increase the signal-to-noise ratio, favouring the signal perception. On the other hand, if that alternative is selected it can prevent low-frequency notes to be copied (Forrest 1994; Slabbekoorn 2004).

The interference by noise makes it harder to detect the signal and also to interpret it (Slabbekoorn 2004), which can limit mate choice, constrain territorial defence, elicit territory shifts, or interfere between mother and offspring coordination (reviewed in Brumm & Slabbekoorn 2005; McLaughlin & Kunc 2013).

To face all these challenges, animals have developed a variety of solutions to avoid this background noise problem (Brumm & Slabbekoorn 2005), starting on the senders side with use signals designed for a better transmission (Klump 1996). Enhancements of signal design and sound production can simply start by a better positioning, depending on the type of habitat and location of the signal recipient (Wiley 1983). However, many more solutions arose to make signals more audible and only those that can stand out will be favoured (Brumm & Slabbekoorn 2005).

Those adaptations can be long-term when involving evolutionary changes in signal transmission as a result of constant noise present for a given period of time (Brumm & Slabbekoorn 2005). An example has been demonstrated by Slabbekoorn and Smith (2002) for differences between songs of male African birds, *Andropadus virens*. The differences they found were related to the habitat: populations from rainforest sang songs with significantly spectral and temporal differences compared to populations from the ecotone forest. More specifically, rainforest songs had lower minimum frequency in two different song types, and for one of them it had also higher maximum frequency and higher rate. The presence of many distinctive noise frequency bands and changes in amplitude in the rainforest may have possibly driven birdsong to change, whereas the fewer distinctive frequency bands and more even amplitude levels ecotone forest didn't request such plasticity (Slabbekoorn & Smith 2002).

Considering the influences of urban noise on the cultural evolution of bird songs, Luther and Baptista studied three adjacent dialects of white-crowned sparrow

(*Zonotrichia leucophrys*) over 30 years in three different areas around San Francisco. With the increase of low-frequency urban noise in the study areas, there was also an increase of the minimum frequency of songs, within and between dialects. Songs with higher minimum frequency were preferred, with one of the dialects being actually replacing another with lower frequency (Luther & Baptista 2009). Nevertheless, habitat-dependent signal differences may be a result of an individual's lifetime plasticity and adaptation to the habitat, instead of a long-term adjustment over time (Brumm & Slabbekoorn 2005).

To overcome background noise, another long-term method used is relying on more than one communication channels at the same time. In case of birds, the use of both acoustic and visual displays may increase their communication effectiveness (Rowe 1999; Brumm & Slabbekoorn 2005). In the nest, vocalizations are masked by the ones from the nest mates, and combining the calls with colourful mouth markings and postures, nestlings increase their chances to stand out (Brumm & Slabbekoorn 2005).

Individuals may also adjust signal traits to manage communication with temporary changes. Short-term adaptations have been studied in greater detail than long-term ones, being the ones that this work will focus on too. Sound-producing animals can temporally adjust the characteristics of their acoustic signals and also its timing (Brumm & Slabbekoorn 2005).

One of the methods of structural adjustment is the regulation of amplitude. Increase signal amplitude, also known as Lombard effect, might come out as being the most obvious way to neutralise noise masking. Singing louder may help the individual not only to be heard by others, but also to hear himself, favouring both production and perception (Brumm & Slabbekoorn 2005). The first evidence of this regulation in the natural habitat was accessed in nightingales (*Luscinia megarhynchos*), where males

sang with higher amplitude in noisier territories than individuals at quieter locations, also singing louder on weekdays than weekends (Brumm 2004). Another study with this same species showed that the individuals increased the mean amplitude of their songs with the increase of the background noise levels, but with a much stronger increase of the low-amplitude notes, in comparison with an also existent but not as strong increase of the sound level of the high-amplitude notes. On a second experiment, playback of different noises were broadcasted and the individuals showed an increase of sound level with white noise, and an even bigger increase with the playback of noise in the spectral region of the individuals songs (Brumm & Todt 2002).

The duration of the signal can also be regulated and it was stated that with an increase of the noise levels to which common marmosets (*Callithrix jacchus*) were exposed, the longer were the call syllables they produced (Brumm 2004). Also, in a period with increase of boat traffic and consequently boat noise, killer whales (*Orcinus orca*) increased the duration of their calls (Foote et al. 2004). The regulation of duration in birds can be achieved in two ways: rate and song duration. With male reed buntings (*Emberiza schoeniclus*), it was found a decrease of the singing rate by males inhabiting noisier places (Gross et al. 2010). Great tits (*Parus major*) decreased song rate only with the white-noise playback and increased the song type bout duration with noise playback experiments (Halfwerk & Slabbekoorn 2009). When exposed to an increasing noise level, the European robin (*Erithacus rubecula*) sang shorter songs (McLaughlin & Kunc 2013), and common blackbirds (*Turdus merula*) from urban sites sang shorter songs with less elements than the ones from the forest sites (Nemeth & Brumm 2009). Another case, with two closely related species, plumbeous vireo (*Vireo plumbeus*) and grey vireo (*Vireo vicinior*), showed opposite results in terms of song duration: plumbeous vireo decreased it, while grey vireos increased the duration of their songs by

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nearly 1.5 times, with noise level increment. In this case study, the song rate of both species was kept uninfluenced by the noise condition (Francis et al. 2011).

According to the mathematical theory of communication, the amount of information of a signal can be maintained with an increase of the redundancy (Shannon & Weaver 1948). Increase serial redundancy by increasing the number of syllables per song is a strategy used by king penguins (*Aptenodytes patagonicu*s) under windy and noisy conditions (Lengagne et al. 1999). On the other hand, chiffchaffs (*Phylloscopus collybita*) had songs with significantly fewer syllables in territories closer to the highway than on the riverside territories (Verzijden et al. 2010).

If the masking by noise is only partial, a change of the spectral parameters might also mitigate the masking (Brumm & Slabbekoorn 2005). Increase spectral characteristics by increasing the peak frequency of song is a strategy used by beluga whales (*Delphinapterus leucas*). When moved to a site with more 12-17 dB of noise level, the individuals increased their peak frequencies to 100-120 kHz and bandwidths between 20 and 40 kHz, when in quieter places those values were 40 and 60 kHz of peak frequency and 15-25 kHz of bandwidth (Au et al. 1985). For birds, studies with common blackbirds showed higher minimum and peak frequencies with higher levels of noise (Nemeth & Brumm 2009; Ripmeester et al. 2010). In a study with common urban species, five out of 12 increased minimum frequencies of songs, but only two of those five increased also the peak frequency (Hu & Cardoso 2010). Many studies in birds have shown an increase of the minimum frequency at noisier locations, with predominantly low-frequency noise (Slabbekoorn & Peet 2003; Slabbekoorn & den Boer-Visser 2006; Slabbekoorn & Ripmeester 2008; Bermúdez-Cuamatzin et al. 2010; Gross et al. 2010; Cardoso & Atwell 2011; Dowling et al. 2012; McLaughlin & Kunc 2013). The frequency shifts can be flexible in a very short time scale: chiffchaffs

increase the minimum frequency of their first ten songs, returning to the pre-noise exposure levels the following day (Verzijden et al. 2010). In a non-manipulated study based on the existing noise levels, it was found an increase of the minimum frequency of the songs in the European serin (*Serinus serinus*) (Mamede & Mota unpublished). On the already referred study with grey and plumbeous vireos, results showed an increase of the minimum frequency with the increase of the amplitude noise levels for the plumbeous and no differences for greys vireos. In terms of high frequency, grey vireos decreased it with the increase of the sound level, and the plumbeous showed no effect on that characteristic (Francis et al. 2011).

The recurrence to different songs with higher minimum frequency when exposed to low-frequency noise, and to song types with lower maximum frequencies when exposed to high-frequency noise is another type of strategy used by great tits (Halfwerk & Slabbekoorn 2009). In another study with the same species conducted in ten big European cities and ten matching forests near those cities, it was found that urban birds sang more often rare and odd song types and less of the typical ones for the species, with also higher minimum frequencies when comparing to the forest birds (Slabbekoorn & den Boer-Visser 2006).

Acoustic signals may lose effectiveness when given simultaneously with others from species inhabiting the same site. By adjusting the singing time by singing asynchronous, birds increase the chances to enhance the distinctiveness of a song (Cody & Brown 1969). Singing a few minutes or seconds later gives individuals the chance to avoid the overlapping with noise (Slabbekoorn 2004; Brumm & Slabbekoorn 2005; Catchpole & Slater 2008). Cody and Brown (1969) found that two commonest bird species sang in an unsynchronized way with each other: wrentits (*Chamaea fuscata*) sang more after Bewick's wrens (*Thryomanes bewickii*) have subsided, also stopping as

soon as the Bewick's wrens begin a new set of songs, producing asynchronous bouts of vocalizations. In other cases, noise level might be loud enough to force animals to stop calling. An example of that has been registered for tawny owls (*Strix aluco*) that stopped calling during rainy nights, even during the courtship period: only 14% and 5% called on two rainy days, whereas 82% and 86% called on two other dry nights (Lengagne & Slater 2002).

There is the need to access the signal-to-noise ratio to make an efficient adjustment of songs (Brumm & Slabbekoorn 2005). More than one regulation can be put together, i.e., the animal can shift the singing time but also increase the amplitude of its song (Brumm & Todt 2004). Common marmosets exposed to increasing levels of noise increased the call syllables and increase also their amplitude (Brumm 2004). Great tits at a noisier location sang longer songs (with more phrases) and with higher minimum frequency (Hamao et al. 2011). Also, the complexity and the several features that compose a song increase the chance of at least a less masked part to still be able to convey the message (Brumm & Slabbekoorn 2005).

To filter the multiple sounds that arrive from multiple sources, receivers also play an active role on the adjustment of signal-to-noise ratios. A distinction between sounds is needed to get the sources that are worth responding to. Although the receiving end might seem passive, there is a variety of solutions to deal with ambient noise (Brumm & Slabbekoorn 2005). The acoustic changes necessary to a better perception by the receiver will depend on the vegetation's density of the surrounding habitat, the distance between signal and noise sources and the receiver, and also the spectral range of those sound sources (Brumm & Slabbekoorn 2005).

Signals and noise have different origins and are transmitted through different pathways. Getting closer to the sender of the signal and/or move away from the noise

source allows the receiver to improve signal-to-noise ratio. Another simple method to improve the audibility is to move the body or head towards the signal, in addition to changing position (Brumm & Slabbekoorn 2005).

When sounds, after passing through all the obstacles, are loud enough to be heard and discriminated, they have to be filtered by the receiver to avoid irrelevant signals. That crucial step is called "auditory scene analysis", i.e., the separation of "meaningful signals and meaningless noise". That separation may also be needed among the overlap of other neighbour species' signals (calls or songs) and intra-specific acoustic variations (Brumm & Slabbekoorn 2005).

1.4. Birdsong and conservation

In 1950, the world population was 2.5 billon people; in mid-2013 that number rose to 7.2 billion; for 2050 it is expected to reach 9.6 billion. With more than half of the world population living in urban areas, urban population reached 3.6 billion in 2011 and is expected to gain more 2.6 billion inhabitants until 2050. With that, over the next four decades, it is expected an "absorption" of almost all of the world population by urban areas (United Nations 2011, 2013). Both increase of population and urban areas are "synonyms", in a large scale, of biodiversity depression (Blair 1996; Turner et al. 2004; Clergeau et al. 2006) and changes in populations' structure, reproduction and behaviour (Clucas & Marzluff 2012). Studies on the impact of highways showed lower breeding densities and lower species diversity adjacent to the roads (reviewed in Slabbekoorn & Ripmeester 2008). When referring to the higher noise levels found in cities, Slabbekoorn and Peet (2003) also found a correlation between the increasing noise levels and lower species richness and density. If there are still other suitable

habitats, anthropogenic noise is a significant factor that drives species to leave cities, highways and other noisy environments (Slabbekoorn & Ripmeester 2008).

With the increase of noise levels, white-crowned sparrows increased the minimum frequency of their songs, between 1969 and 2005. Testing the response to 1969 and 2005 songs, males responded more strongly to the latest, demonstrating that vocal adjustments can affect communication and song's cultural evolution (Luther & Derryberry 2012). Young birds, when arriving to a noisier place and copying the less masked songs from their neighbours may lead to the extinction of other songs masked in a higher level (Slabbekoorn & den Boer-Visser 2006). Changes in a sexually selected trait like song, essential on mate choice circumstances, may then bring serious consequences on the reproductive success of the specie and consequently on species' density (McLaughlin & Kunc 2013).

1.5. Study species

The European serin, *Serinus serinus,* is a passerine that belongs to the Fringillidae family, Carduelinae sub-family, which includes 207 species (Lepage, D., 2011; Oiseaux, 2011). It is a small socially monogamous, non-territorial, resident and gregarious bird with around 11.5 cm long, short and forked tail, bill very short, conical and swollen at the base (Mota 1995; Cramp 1998). With sexual dimorphism, the male presents a "brilliantly yellow" forehead, throat and breast, and the female has a duller yellow colour. Juveniles resemble females but even duller (Cramp 1998).

Very common in Southern Europe, this species is geographically spread, inhabiting a big variety of sites, from forest edges, to cultivated fields, gardens and urban areas. It is highly tolerant to human presence, preferring areas with an adequate

amount of resources. Their diet consists of seeds and other parts of the plant, also eating small invertebrates occasionally (Cramp 1998).

Males sing virtually all year, with more intensity in the breeding season, from April to August (Géroudet 1957 in Cramp 1998), which has great impact on female nest-building stimulation (Mota & Depraz 2004). At that period males typically display alone, from tall exposed tree or overhead wires. Song activity decreases throughout incubation and chick-rearing and outside the breeding season, males sing especially from mid-winter onwards (Cramp 1998). Serins also produce alarm calls, often in presence of predators or intruders (Mota & Cardoso 2001).

Serin song is a high-pitched and fast rambled sum of indistinctive elements, easily perceived by the Human ear. Phonetically, it has a broad frequency range of about 8 kHz and a mean frequency of maximum intensity of 6.3 kHz (Mota & Cardoso 2001). The last value goes against the expected frequency of maximum intensity for serin body weight, i.e., for 10.4 g of weight (Cramp 1998) serin should have a frequency of 4 kHz, according to Wallschläger's regression function of "centre frequency" (Wallschläger 1980). The possible conclusions to that phenomenon are that serins are less restricted by the rate/bandwidth constraint, that they sing closer to their physiological limit or they are particularly adapted to singing (Mota & Cardoso 2001).

Syntactically, serin song has two modes of production: a repetitive and a sequential mode. The first one is a simpler mode formed by repeated or smaller elements forming trills, frequent at the beginning and in the middle of songs. The sequential mode is larger and the predominant part of most songs (and by that, the most distinguishable part of the song to the human ear) (figure 2). It is characterized by a fast succession of different and mostly non-repeated syllables, sung in a determined order. In comparison with the repetitive mode, the elements that form syllables are tightly

packed, which results in a higher number of intensity peaks per unit of time (Mota & Cardoso 2001).
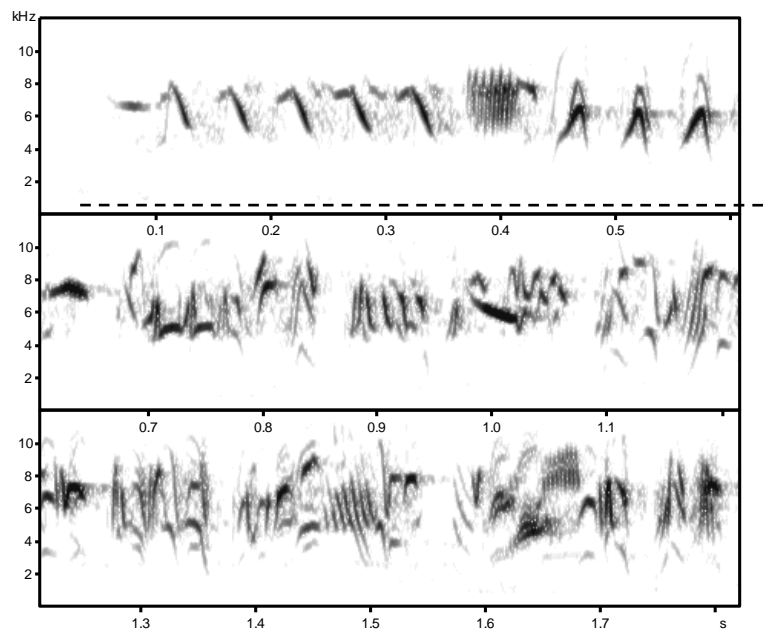
Figure 2. Spectrogram of part of a serin song. It starts with syllables in repetitive mode (marked by the horizontal dashed line) arranged in three consecutive trills and then follows with the sequential mode. The axes indicate the frequency (kHz) and time (s).

Song elements are organized in a long circular sequence, sung in a stereotyped way with very little variation between songs. However, sequences can cycle back after some time, merging in different points of the cycle and creating a song that look "doubled". As a cycle, a longer song is allowed and maintained in comparison to the longitude that the repertoire alone allows. On average, song duration is around 3 seconds and the syllable repertoire has around 63 syllables (Mota & Cardoso 2001; Mamede & Mota 2012).

Birds' repertoires recorded in the same location and year present more similarities that the ones from birds from different places or years, suggesting a geographical variance in the repertoire (Mota & Cardoso 2001). In terms of variation

within individuals' repertoire, male serins exhibit very limited vocal plasticity between years. The repertoire size did not increase through the time of the experiment and it did not appear to be an age indicator (Mamede & Mota 2012).

1.6. Objectives

The objective of this study was to evaluate the capacity of male serins to adapt their songs to urban noise. Although research on the impact of noise has greatly increased in the last decade, studies on the impact of noise on non-territorial birds are scarce or non-existent. Serins are non-territorial which make them a good subject for this type of studies. There is only one study, to our knowledge, on the impact of noise in this species that refers that serins sang for longer periods during week days, in areas with higher anthropogenic noise, decreasing its vocal activity during weekends. Nevertheless, structural modifications in songs under noise masking remain unknown.

Great tits showed structural plasticity switching to song types with higher minimum frequencies when exposed to low-frequency noise and switching to song types with lower maximum frequencies when exposed to high-frequency noise. That flexibility also served as base to this work because, although the European serin does not have different song types, changes in minimum and maximum frequencies could be performed with masking by low and high-frequency noise. To accomplish that purpose, two types of experimental playbacks were created: low and high-frequency stimulus.

The working hypothesis then were that, with noise stimuli: (1) individuals would change songs' syntax, (2) they would reduce the time spent singing and (3) low and high-frequency stimulus would influence the song in opposite ways: minimum frequencies would increase with exposure to low-frequency noise and maximum frequencies would decrease with high-frequency noise.

Chapter 2   **Material and Methods**

2.1. Study area

Experiments were carried out in April and May 2013, between 07:30am and 14:00pm, in the urban and suburban area of Coimbra, Portugal, as well as in rural areas around Coimbra. Specifically, data from urban areas was collected in the City Park, Jardim da Sereia and Santa Clara; and in suburban areas such as Escola Superior Agrária de Coimbra, University of Coimbra Astronomical Observatory and Hospital Centre of Coimbra. For rural areas, recordings were made in the surrounding areas of Pereira, Arzila and Figueiró do Campo. Urban, suburban and rural points are represented in figure 3 and GPS coordinates in Appendix I - A.
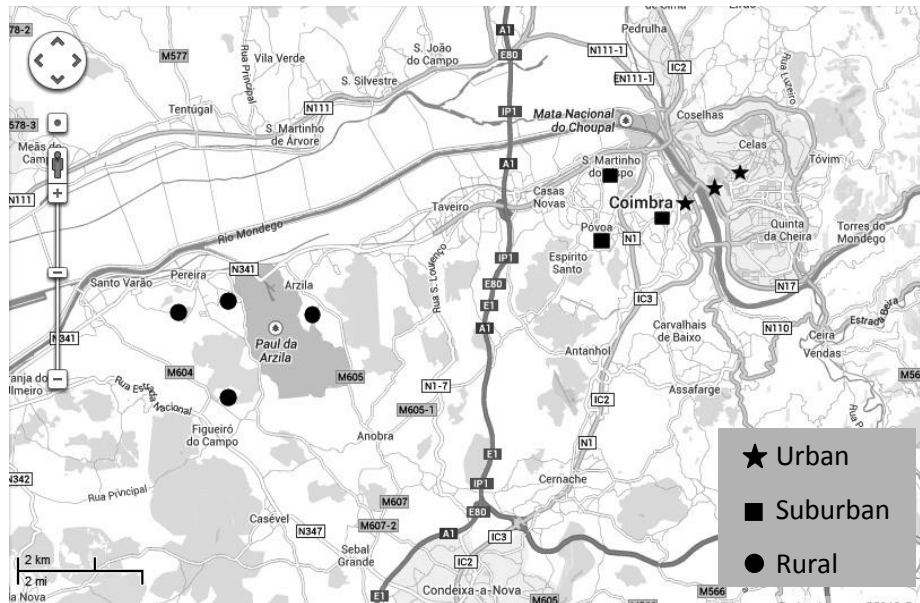
Figure 3. Study area with urban, suburban and rural points where data was collected.

2.2. Noise stimuli and experimental design

Two noise stimuli of one minute each were digitally created using Avisoft SASLab Pro (version 5.2.06, R. Specht). The low-frequency stimulus was created by

applying a low-pass FIR filter to white noise, "Hamming" window, with a cut-off frequency at 3000 Hz. The high-frequency stimulus was created by applying a band-pass FIR filter from 10000 to 12000 Hz, "Hamming" window. Both types of stimuli were created to overlap serin song in 1 kHz, value set as being adequate to provoke an alteration in the singing behaviour of the subjects. A modulated fade in of sine ½ was applied for the first five seconds of noise and amplitudes were standardized at 90%. Using a sound level meter Tenma 72-860A, the stimuli amplitude was settled at ±65dB at 10 meters distance (which is approximately the distance between the bird and the stimulus), in order to standardize sound amplitude for each focal bird.

In the field, after identifying visually and/or acoustically an isolated male, the researcher would as fast and as silent as possible approach the bird, forming a triangle with 10m sides, with the bird, the speaker and the microphone in the vertices (figure 4).
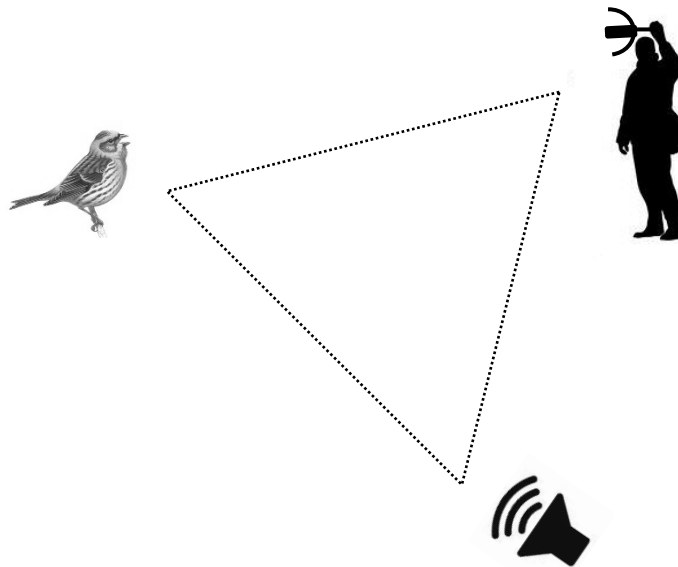
Figure 4. Representation of the triangular formation between the bird, microphone and speaker.

Each experiment consisted of three distinct phases. The first entailed a minimum of five songs sung by the male, called **phase before stimulus**. The **phase during**

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stimulus comprised the playback of one minute noise playback. The third and last phase, the **phase after stimulus**, also consisted of a minimum of five songs. As each individual was recorded before, during and after stimulus, it served as control for itself. It was aimed to expose at least 15 individuals to each type of stimuli. Since some subjects did not stay and sing for a long period of time, we attempted to record a minimum of five songs (or up to one minute) before and after stimulus, in order to consider the test valid. This minimum number of songs was defined because it allowed to efficiently extract the parameters for posterior analysis. If the bird changed his singing position during the experiment we would redirect once the speaker and the microphone. If the bird moved a second time or flew away we discarded the experiment. Also birds that did not sing or stay throughout the three phases were discarded. Serins are a non-territorial species, but during the reproductive season they sing at preferred posts, particularly near their nests (Mota 1999). Adding to that, experiments performed at close locations were avoided and, through spectrogram inspections, it was check if the recorded songs belonged to other previously recorded males, since song structure and syllable repertoire are reliable characteristics used to distinguish different individuals (Mota & Cardoso 2001; Mamede & Mota 2012).

Recordings were made using a portable digital recorder Marantz Professional PMD661 connected to a Telinga parabolic microphone PRO 8 "twin science". The microphone was switched to "filtered, with separated channels" option (stereo recording and attenuation of - 20 dB). The stimuli were played by a speaker Samson Expedition P40i connected to an mp3 player Sony NWZ-E453. The speaker was placed 25cm from the ground and directed towards the singing bird so that noise exposure conditions between experiments would stay as constant as possible. There were two researchers involved in every experiment: one did the recording and the other performed the

playback of the stimulus. The background noise at the vertical of the bird's position was measured after each experiment with the sound pressure level meter, in order to have a measure of the background noise the subject was already exposed without our stimulus.

2.3. Song analysis

A total of 81 individuals were recorded but only 34 individuals were considered for analysis because they kept on singing for the three phases of the experiment. From those 34, 15 were exposed to low-frequency and 19 to high-frequency noise. Sound analyses were made using the software Avisoft SASLab Pro (version 5.2.06, R. Specht). All recordings were converted to a frequency of 24000 Hz and spectrogram parameters used were "Hamming" window, FFT = 512, frame = 50% and overlap = 93.75 %, which result in bandwidth = 22 Hz, resolution = 47 Hz and temporal resolution = 1.33ms. A high-pass and a low-pass filter would be used in the spectrogram to eliminate low and high-frequency noise, respectively.

For each individual, for the three phases, the temporal parameters analysed were song duration and interval between songs, both measured in seconds (Appendix II − A and B). Songs were labelled by hand so we could obtain the duration values for both parameters with the automatic tools from the software. It was analysed the total number of syllables that formed trills (repetitive mode) per song for the three phases, for both groups. These last parameters were taken by visual inspection of each song.

A selection of syllables and sequences of syllables (called "sections" from now on) was made to test for changes in frequency. A search for repeated syllables and sections was performed by visual inspection for phases before, during and after stimuli. Values of section duration (s) and peak frequency (frequency of maximum amplitude,

measured in Hz) of songs, syllables and sections were taken automatically. For greater accuracy of the values given by the software, songs, sections and syllables were labelled with a hand-adjusted marker (figure 5). Because the quality of the recordings did not always allow syllables and sections identification, a subset of 14 individuals was used for high-frequency group and for low-frequency group, 18 individuals were used for syllables and 10 individuals for sections. Besides the good quality required, sections and syllables selection also had to fulfil the following criteria: (1) for low-frequency playback recordings, sections had to be constituted mainly by low-frequency syllables and the individual syllable chosen had also to of be low-frequency; (2) for high-frequency recordings, sections with mainly high-frequency syllables and individual high-frequency syllables were chosen; and (3) sequences and syllables chosen had to be repeated at least twice in each phase (before, during and after stimulus). The first and second criteria' frequency preferences were set so that serin song was as close as possible to the stimulus so the probability of occurring changes would be higher.
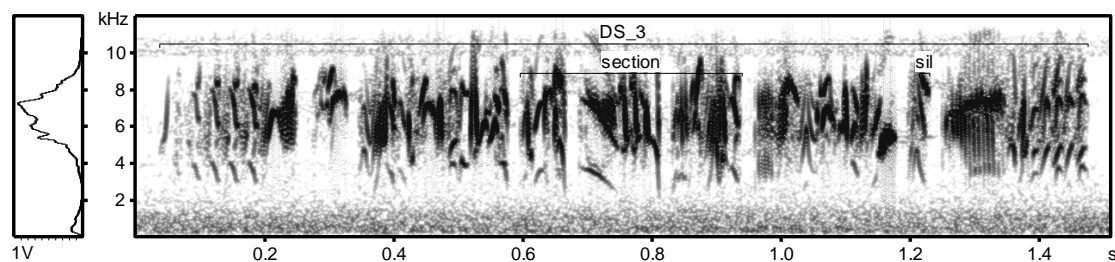
Figure 5. Spectrogram of a serin song with labels of song ("DS_3" - third song during high-frequency stimulus), "section" and syllable ("sil"). The axes indicate the frequency (kHz) and time (s). The amplitude spectrum is represented on the left (V). The peak frequency of song and section (that in this particular case had the same value) is represented by the dashed white line.

Mean values for song, interval and section duration were calculated, and total number of syllables from trills of all songs per phase, while the mean value for peak frequency of songs, sections and syllables was calculated for five songs sung in each one of the three phases. There was an exception for two individuals that only sang three (subjects 8 and 11) and other two that only sang four songs (individual 7 and 18) in one of the phases (Appendix III – A and B).

2.4. Statistical analysis

Statistical analysis was carried out in IBM SPSS Statistics ® 20 software.

To analyse all the song parameters it was verified the normality through a Kolmogorov-Smirnov test. All the variables had normal distribution except the interval between songs, which was then transformed with the Log_{10} function. Also the number of syllables per trill and peak frequency of songs did not have a normal distribution but since they are discrete variables, they were analysed using Poisson distribution.

A Generalized Linear Model with repeated measures was performed to check for variance of each song variable for the three phases of the experiment (before, during and after stimulus), with the individuals as subjects and phase as the within-subject variable. The background noise was set as predictor to control for its influence on the experiment. This analysis was performed for low and high-frequency groups separately.

For all tests the significance was two tailed and $p < 0.05$.

Chapter 3   Results

3.1. Differences between phase before, during and after stimulus

Individuals exposed to the low-frequency playback showed a significant variation in section duration (χ^2_2=8.223, P=0.016) (table II) and in the peak frequency of section (χ^2_2=7.146, P=0.028) for phases before, during and after stimulus. Both section duration and peak frequency decreased throughout the three phases. All the other variables: song, interval and section duration, as well as peak frequency of song and syllable, revealed no influence from the stimulus, without significant changes between the three phases.

Table II. Variation of song parameters between phase before, during and after stimulus, for low-frequency group.

Song parameter	n	Phase	Mean ± Std. Error	χ^2	df	Sig
Song duration (s)	19	Before During After	4.425 ± 0.272 4.138 ± 0.256 3.721 ± 0.353	0.920	2	0.631
Interval duration (s)	19	Before During After	0.533 ± 0.087 0.524 ± 0.031 0.569 ± 0.069	2.542	2	0.281
Number of syllables per trill	19	Before During After	14.670 ± 2.432 13.890 ± 2.389 13.780 ± 2.308	0.074	2	0.963
Peak frequency song (Hz)	19	Before During After	6414.890 ± 118.181 6384.890 ± 134.153 6363.560 ± 153.634	0.052	2	0.974
Peak frequency syllable (Hz)	18	Before During After	3946.610 ± 126.721 3942.610 ± 132.293 3923.610 ± 123.029	0.211	2	0.900
Section duration (s)	9	Before During After	1.063 ± 0.042 1.058 ± 0.043 1.052 ± 0.042	8.223	2	0.016
Peak frequency section (Hz)	9	Before During After	6320.560 ± 189.987 6134.000 ± 153.703 6120.560 ± 170.539	7.146	2	0.028

In the experiment of the high-frequency noise it was not found significant variation in all the variables with masking by noise, between the three phases (table III). However, peak frequency of song and syllable showed a non-significant tendency (song: χ^2_2=5.289, P=0.071; syllable: χ^2_2=5.072, P=0.079). That tendency shows that both peak frequencies increased in phase during stimulus and decreased again in phase after stimulus. Nevertheless, the size of this group is relatively small so a larger sample would be needed either confirm that tendency as significant or to denote no impact from noise on high-frequency group.

Table III. Variation of song parameters between phase before, during and after stimulus, for high-frequency group.

Song parameter	n	Phase	Mean ± Std. Error	χ^2	df	Sig
Song duration (s)	15	Before	3.617 ± 0.372	2.439	2	0.295
		During	3.905 ± 0.308			
		After	3.574 ± 0.312			
Interval duration (s)	15	Before	0.479 ± 0.062	4.131	2	0.127
		During	0.558 ± 0.048			
		After	0.569 ± 0.049			
Number of syllables per trill	15	Before	14.530 ± 2.649	0.438	2	0.803
		During	13.470 ± 1.833			
		After	13.800 ± 2.357			
Peak frequency song (Hz)	15	Before	6299.600 ± 96.099	5.289	2	0.071
		During	6363.000 ± 85.711			
		After	6198.400 ± 50.833			
Peak frequency syllable (Hz)	15	Before	8493.200 ± 133.688	5.072	2	0.079
		During	8747.070 ± 137.469			
		After	8571.130 ± 175.564			
Section duration (s)	14	Before	0.874 ± 0.070	2.385	2	0.303
		During	0.711 ± 0.071			
		After	0.876 ± 0.069			
Peak frequency section (Hz)	14	Before	6335.430 ± 136.758	0.693	2	0.707
		During	6235.790 ± 114.336			
		After	6288.360 ± 114.913			

Chapter 4   **Discussion**

These experiments provide an experimental evidence of modifications in the singing behaviour of male serins when exposed to noise playbacks. Although the song in this species is very rigid (Mota & Cardoso 2001), there appears to exist some temporal and structural noise-dependent regulations. Individuals exposed to low-frequency noise compressed the sections of their songs, decreasing their duration when masked by noise. That change made songs slightly faster (with more syllables per second). Individuals also decreased the peak frequency of those same sections, between the three phases. That decrease took place without any syntactical modification since comparisons were made between the same sections, composed by the same syllables. For all the other variables analysed, individuals from low-frequency group showed no significant variance during the three phases of the experiment.

Subjects exposed to high-frequency stimulus did not show any significant variation between phases. However, they showed variation close to significance in variables such as peak frequency of songs (p=0.071) and peak frequency of syllables (p=0.079), increasing both values in the phase during noise and decreasing them again in the phase after stimulus.

4.1. Effects of low-frequency noise in birdsong

Noise present in cities and also the natural noise present in more rural areas is mainly low-frequency, with most energy under 2 kHz (Forrest 1994; Slabbekoorn 2004). Several studies in natural conditions comparing sites with lower background noise levels with noisier places have shown frequency changes in songs masked by noise, especially by increasing minimum frequencies: in great tits (Slabbekoorn & Peet 2003), song sparrows (*Melospiza melodia*) (Wood et al. 2006), common blackbirds

(Nemeth & Brumm 2009), common chiffchaffs (Verzijden et al. 2010), white-crowned sparrows (Luther & Derryberry 2012), the European robin (McLaughlin & Kunc 2013) and house wrens (*Troglodytes aedon*) (Redondo et al. 2013). Those evidences were also shown in experimental studies using noise playbacks in male great tits (Halfwerk & Slabbekoorn 2009) and reed buntings (Gross et al. 2010). The present study concerns an experimental trial with a non-territorial bird with a very complex song that presented several difficulties on the measurement of minimum and maximum frequencies. Those aspects are referred in a later section.

A study performed with the European serin (Díaz et al. 2011) has shown that males sang for longer periods of time during week days in areas with higher anthropogenic noise, but decreasing sharply above the 70 dB threshold. This threshold suggests that this type of strategy must be costly. In the same areas, males significantly decreased vocal activity (proportion of time spent singing) during weekends when noise levels were lower in comparison to week days, except in areas with background noise between 50 and 70 dB. A higher vocal activity in noisier places implies more costs to the singing male and also implies a trade-off between vigilance and daily activities and singing behaviour. Díaz *et al*. did not check for vocal responses to noise as in our study, and only considered time budget changes on singing time and singing effort. It is, thus, difficult to establish comparisons between the two studies, as they were essentially measuring different things in response to noise.

As previously stated, there was an acceleration of sections of songs performed by males stimulated with low-frequency noise in this experiment. That modification might bring negative consequences in female mate choice since female serins showed preference for slower songs, produced by adding 10 ms to the inter-syllable intervals of original serin songs (Cardoso et al. 2007). The reduction of intervals between syllables

and the consequent increase of syllable rate was interpreted as an aggressive signal for females, inhibiting their vocal responses. The acceleration of sections in the present noise experiments might have increased song rate too, possibly leading to an unfavourable interpretation by females. That same speeding of sections can also provoke aggressiveness in males. In another study involving overlapping playbacks of serins' songs, half of subjects responded aggressively by approaching the speaker and by increasing song duration and syllable rate (Funghi et al. 2013 submitted to Animal Behaviour). There are three relevant criteria to state a signal as aggressive: (1) whether there is an increase of the signal in aggressive context (context criterion); (2) if the signal is a predictor of signaller' aggressive escalation (predictive criterion); and (3) whether there is a response by the receiver of the signal (response criterion) (Searcy & Beecher 2009). The increase of sections with noise in this experiment, can therefore by interpreted as aggressive because it possibly increased syllable rate (first Searcy and Beecher's criterion fulfilled) and because females would probably respond negatively to those modifications (which fulfils the third criterion). Song duration was not affected by noise in the current experiment.

Males from low-frequency group also changed the peak frequency of their sections, decreasing it. That modification might have been unfavourable due to the fact that females prefer songs with higher frequencies (produced by shifting peak frequency upwards in 1 kHz) (Cardoso et al. 2007). Nevertheless, that negative consequence is not likely to happen because the peak frequency of the entire song did not suffer significant alterations with noise playback, during the three phases of the experiment. One possible explanation for this decrease in peak frequency of the sections might be related with the acceleration they suffered. Acceleration means reducing the intervals between syllables, where mini-breaths are performed by the bird to sustain long songs (Hartley & Suthers

1989). This method suggest that syllables are a product of coordinated of motor outputs to syringeal and respiratory muscles. Nevertheless, the cost of performing those mini-breaths in fast syllables will make it harder to maintain long and uninterrupted songs (Suthers & Goller 1997). If these mini-breaths are reduced, birds may incur in a cost. It could be argued that this cost is reflected in the animal having to put the maximum of energy at lower frequencies as it is probably more costly to sustain that energy at higher frequencies. Peak frequency changes performed by other species were investigated in grey shrike-thrush (*Colluricincla harmonica*) and common black birds in sites with higher levels of noise (Parris & Schneider 2008; Nemeth & Brumm 2009; Ripmeester et al. 2010). Results from Parris and Schneider (2008) point that grey shrike-thrush (*Colluricincla harmonica*), a species with lower frequency (range: 1.5 - 4 kHz), sang at higher peak frequency with traffic noise, while grey fantail (*Rhipidura fuliginosa*; frequency range: 4 - 7.5 kHz) did not show frequency changes with noise. However, other species showed increases of the minimum frequencies when masked by noise, but no changes in peak frequency and spectral energy (reviewed in Cardoso & Atwell 2011). Peak frequency is the frequency of highest volume and individuals might have adopted changes in more microscopic parameters such as minimum and/or maximum frequencies instead. Those parameters were considered for analysis but could not be measured in a direct way as in other studies, due to the complexity of serin songs and to the overlap of noise in this experiment. Those methodological aspects are discussed in a section ahead.

A study with 529 different bird species (passerines and nonpasserines) comparing urban and nonurban habitats (Hu & Cardoso 2009) has shown that urban bird' songs or vocalizations had higher peak frequencies than their congeneric nonurban species, and urban passerines had higher minimum frequencies in comparison with the

nonurban individuals. Different sound frequencies used by different species can contribute to shape birds communities inhabiting urban areas.

Amplitude was not measured in the present experiment because it is extremely difficult to do it in the field: it depends of many factors such as wind, temperature, distance to the signaller and direction to which it is singing. Nightingales at natural noisier locations sang with higher amplitude than males inhabiting places with lower levels of noise, result also confirmed with noise playbacks (Brumm & Todt 2002; Brumm 2004). These results suggest that males sing louder with higher levels of noise, in order to be capable of exchanging information between individuals under constrained conditions. Nevertheless, there are energetic costs adjacent to the increase of amplitude, as well as compromises with other factors such as detection of predators.

Although it was not attempted to take measurements of syntax, the variation in the number of syllables per trill present in songs was recorded in this experiment. Subjects showed no variation in that parameter throughout the phases of the experiments. A study from Podos (1997) analysed trilled vocalizations from 34 species of songbirds, and particularly trill rate and frequency bandwidth. What he found was that frequency bandwidth decreased with the increase of trill rates, and that trills with low rates had wider variance in frequency bandwidth, being the inverse also true to high trill rates. His results suggest that there are performance constrains present in trills that limited its evolutionary diversification (Podos 1997). The costs of trills' performance and its function are unknown in the serin. With that, the occurrence of changes in this fast succession of syllables might be due to their function in the song, and not due to the impact of noise. Structural changes of this type might be very difficult to perform and might not be worthy either, i.e., the energetic cost needed to make those alterations might not compensate the outcome.

4.2. Effects of high-frequency noise in birdsong

The results from this experiment showed no modifications in the group exposed to high-frequency stimulus. Nevertheless, they showed variation close to significance in peak frequency of songs (p=0.071) and peak frequency of syllables (p=0.079). Specifically, both peak frequencies increased during the exposure to noise and decreased again in the phase after stimulus. The peak frequency of serin song is around 6.3 kHz (Mota & Cardoso 2001) and low-frequency stimulus range was 0 - 3 kHz and high-frequency stimulus range was 10 - 12 kHz. Both types of stimuli were created to overlap serin song in about 1 kHz (figure 6). With that, the frequency range from the low-frequency stimulus overlap to the peak frequency of serin song was 3 kHz, while the distance from the peak frequency of song to the high-frequency stimulus overlap was 4 kHz. For that reason, the impact of the high-frequency playback might have been smaller in male songs. Moreover, high frequencies are more easily attenuated and degraded in natural environments (Wiley & Richards 1982; Wiley 1983; Slabbekoorn 2004), which might have decreased the impact of the high-frequency noise at the individual level.
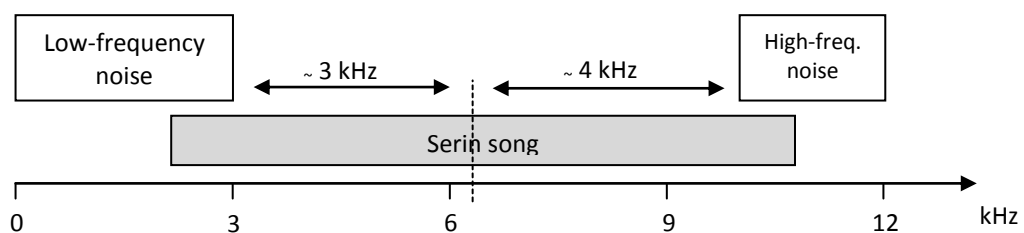
Figure 6. Representation of frequency ranges: serin song (grey box), low-frequency stimulus and high-frequency stimulus. Frequency overlap between song and low and high-frequency stimuli is similar, but the distance from the peak frequency of song (vertical dashed line) is greater for the high-frequency stimulus. The axis represents frequency (kHz).

One study involving high-frequency stimulus playbacks was performed with great tits to test for relationship between noise and song frequency use (Halfwerk & Slabbekoorn 2009). They exposed male birds to both low and high-frequency noise. As results, songs types with higher minimum frequencies were sung for longer periods of time when exposed to low-frequency noise, whereas song types with lower maximum frequencies were sung for longer with high-frequency stimulus. Serins do not have different song types but the maximum frequencies of their songs might also be affected by high-frequency noise. For that, there is a need to increase the sample size of individuals exposed to high-frequency stimulus in this experiment. Variations in peak frequency of song and syllables were close to significance in the results. An increase in sample size is crucial to either confirm if there is a significant variation or not in these song parameters.

4.3. Methodological aspects

With serin's intricate and mostly non-repeated syllables, measures of minimum frequencies become very difficult, being hard to accurately extract values. By changing contrast levels of the spectrogram in Avisoft, changes in measurements of minimum frequencies also occur (Zollinger et al. 2012) (figure 7). Adding to that constraint, in experiments with noise playbacks there is, most of the time, an overlap between songs and noise, adding another challenge to correctly measure minimum frequencies. Figure 7 visually represents both problems. In the particular case of this experiment, where there was also a group of individuals exposed to high frequency stimulus, that difficulty was present in high frequency measurements as well.
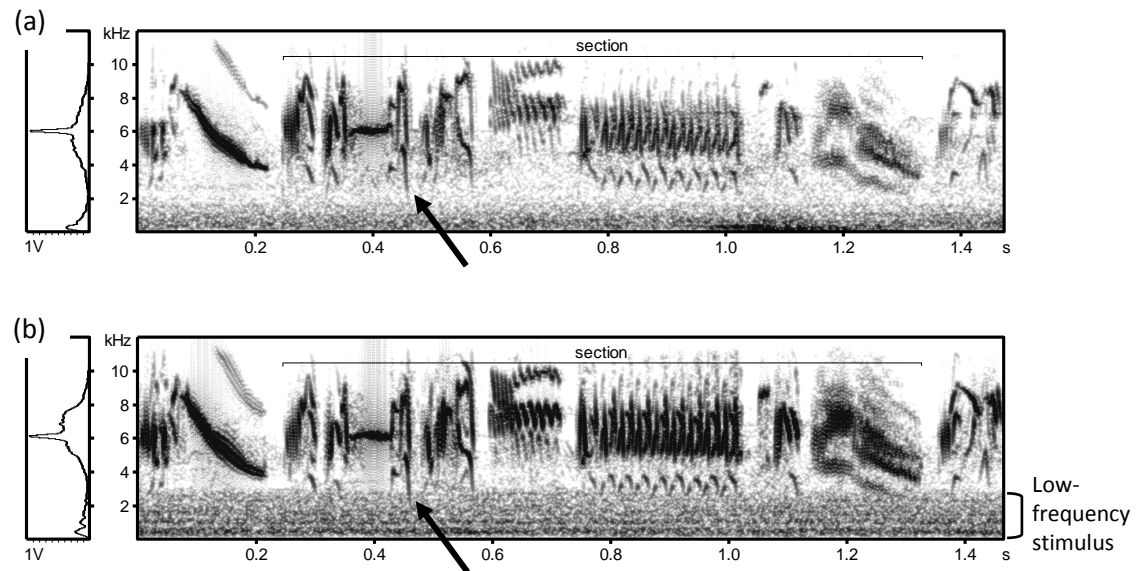
Figure 7. Spectrograms of segments of songs in different phases of the experiment: (a) phase before and (b) phase during low-frequency stimulus. Spectrogram (b) has higher contrast than spectrogram (a). The syllable pointed by the arrows in the two different songs exemplifies how changes in the contrast can also cause visual changes of the minimum frequency. The overlap of noise stimulus over the same syllable in (b) reduces as well the accuracy of measurements. The axes indicate the frequency (kHz) and time (s). The amplitude spectrum is represented on the left (V).

An alternative measurement suggested in the literature to measure minimum frequencies is through power spectrum (Zollinger et al. 2012). However, that method is only efficient in analysis of clean recordings, i.e., recordings with high signal to noise ratios or when noise does not overlap the frequencies of the birds in study. The following example, represented in figures 8 and 9, represents the limitations of the power spectrum measurements along with spectrogram analysis. Measuring minimum frequencies visually on the spectrogram of a song randomly selected for this example, it is possible to visualize that the minimum frequency of one of the syllables is close to 2 kHz (pointed by the arrow in figure 8).
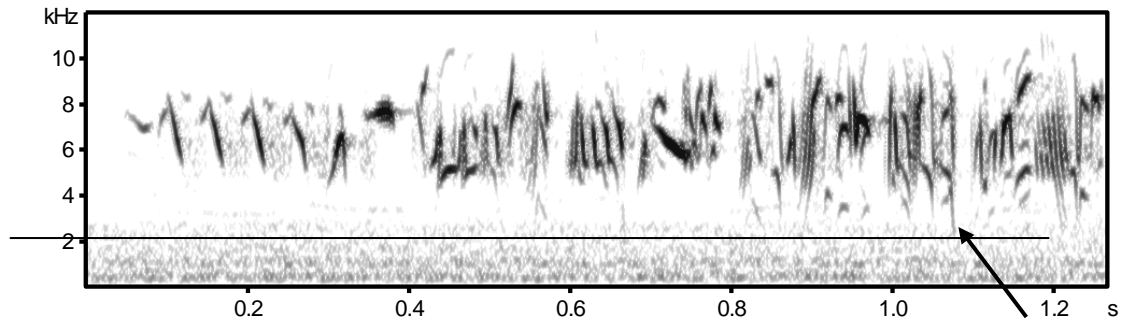
Figure 8. Spectrogram of a serin song in the phase during low-frequency stimulus. The arrow shows a syllable whose minimum frequency is ~2 kHz (checked by the horizontal line). The axes indicate the frequency (kHz) and time (s).

The analysis of the minimum frequency with a power spectrum of the same song during noise exposure revealed that it was also not possible to accurately extract values due to the noise overlap with the serin song. Minimum frequency of the same song measured in the power spectrum would be around 3 kHz (vertical line in figure 9), differing in approximately 1 kHz from the value given by the previous spectrogram (figure 8).

Hu & Cardoso (2009) refer that minimum frequencies adjustments might be easier and more subtle than changing the peak frequencies. That is one possible interpretation for the fact that subjects exposed to noise in the current experiment showed no changes in peak frequencies of song. To control for this limitations of minimum and maximum frequencies measurements, syllables and sections of songs were chosen. Both parameters chosen had mainly low and high-frequencies, in case of exposures to low or high-frequency noise respectively, and so that the probability of the occurrence of changes with stimuli would be higher. From those sections and syllables values of peak frequency were taken, once measurements of peak frequency are not

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affected by the presence of more or less noise. But then again, these syllables or elements might be too low or too high for the effect to be detected in low and high-frequency noise exposures, respectively.
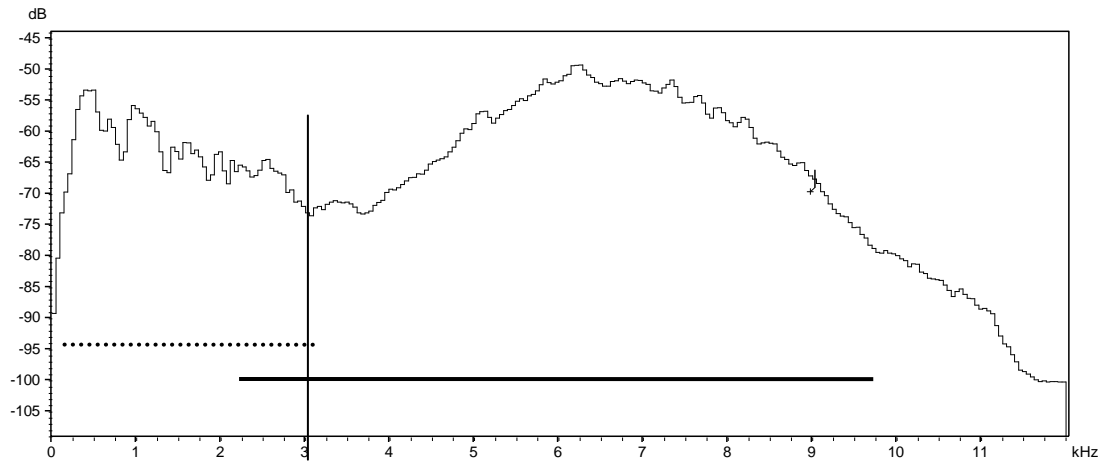
Figure 9. Power spectrum of the same serin song represented in the previous spectrogram (figure 8), also during low-frequency stimulus. The dashed horizontal line represents the noise stimulus; the continuous horizontal line embodies the serin song; and the vertical line the minimum frequency that would be extracted analysing just this power spectrum. Both horizontal lines are overlapped due to the minimum frequency of ~2 kHz minimum frequency given by the preceding spectrogram. The axes indicate the amplitude (dB) and frequency (kHz).

4.4. Final remarks and future work

Anthropogenic noise, as stated throughout this thesis, may affect birds in many ways. Changes in the perception of an acoustic signal and general interference in the relationships between individuals can bring serious consequences for birds. Gross *et al.* (2010) reviews several mechanisms that can influence reproductive success, in

situations with noise masking: (1) increase of minimum frequency reduces song effectiveness because higher frequencies are more easily attenuated (Wiley & Richards 1982); (2) changes in minimum frequencies may reduce efficient recognition from the conspecifics (Nelson 1988, 1989); (3) noise can change female preferences (Swaddle & Page 2007); (4) males may avoid noisier areas; (6) noise may reduce reproductive success by affecting vigilance and feeding behaviour (Quinn et al. 2006). Impact of noise also affects species richness, bird abundance and diversity (Reijnen et al. 1995; Rheindt 2003; Clergeau et al. 2006; Proppe et al. 2013). Those values were found to be lower near motorways, a phenomenon that can be largely explained by noise levels: reduced density of birds was found in noisier areas. Male willow warblers (*Phylloscopus trochilus*) inhabiting territories closer to a highway had difficulties in attract and keep females, moving from those territories in the next year. One of the possible reasons pointed to justify that dispersal behaviour was the song distortion caused by high noise levels (Reijnen & Foppen 1994). In another research with seven different species, overall richness declined in areas with higher noise levels and the abundance of three of the seven species was reduced in noisier locations (Proppe et al. 2013). Urban birds often experience high levels of noise (Slabbekoorn & den Boer-Visser 2006) contributing to a loss of diversity and to a potential homogenization of birds' populations (Clergeau et al. 2006).

With all the adjustments performed to overcome noise, there is a potential of emergence of new urban species over an evolutionary time scale (Slabbekoorn & Ripmeester 2008). Many physiological (frequency alterations, adjusted stress responses) and ecological (nesting type, environmental tolerance) factors were identified as contributors toward urban living (reviewed in Hu & Cardoso 2009).

Although there are not many adjustments performed by the subjects on this experiment, males showed some temporal and structural flexibility, at least with the most common type of noise: low-frequency stimulus. That flexibility in this species might help it thrive in places with high anthropogenic noise.

The spring of 2013 was very rainy, windy and cold, which added many difficulties to the efficient recording of birds in natural conditions. Bad weather prevented many field expeditions and recordings and also had an impact on birds behaviour: serins stay near their nests during reproductive season (Mota 1999), but the fact that this year's temperature was very low could have delayed the start of pairing. In future research, an increase of the study sample is required to confirm differences between the types of stimuli used and to possibly obtain significant variances in more parameters between the three phases of the experiment. Signal-to-noise ratios should also be improved, i.e., the overlap of noise over songs should be decreased. That alteration must especially be done with the low-frequency stimulus, because the overlap with syllables with lower minimum frequencies was higher than the overlap with the high-frequency stimulus. Reducing the frequency range of the low-frequency stimulus might then improve future measurements of recordings.

References

Au, W. W. L., Carder, D. A., Penner, R. H. & Scronce, B. L. 1985. Demonstration of adaptation in beluga whale echolocation. *Journal of the Acoustical Society of America*, 77, 726-730.

Bermúdez-Cuamatzin, E., Ríos-Chelén, A. A., Gil, D. & Garcia, C. M. 2010. Experimental evidence for real-time song frequency shift in response to urban noise in a passerine bird. *Biology Letters*.

Blair, R. B. 1996. Land use and avian species diversity along an urban gradient. *Ecological Applications*, 6, 506-519.

Brackenbury, J. H. 1982. Production, Perception, and Design Features of Sounds. In: *Acoustic Communication in Birds* (Ed. by D. E. Kroodsma & E. H. Miller), pp. 53-73. New York: Academic Press.

Bradbury, J. W. & Vehrencamp, S. L. 1998. *Principles of animal communication*. Sunderland: Sinauer Associates.

Brumm, H. 2004. The impact of environmental noise on song amplitude in a territorial bird. *Journal of Animal Ecology*, 73, 434-440.

Brumm, H. & Slabbekoorn, H. 2005. Acoustic Communication in Noise. In: *Advances in the Study of Behavior* (Ed. by C. T. S. T. J. R. H. J. B. Peter J. B. Slater & N. Marc), pp. 151-209: Academic Press.

Brumm, H. & Todt, D. 2002. Noise-dependent song amplitude regulation in a territorial songbird. *Animal Behaviour*, 63, 891-897.

Brumm, H. & Todt, D. 2004. Male-male vocal interactions and the adjustment of song amplitude in a territorial bird. *Animal Behaviour*, 67, 281-286.

Cardoso, G. C. & Atwell, J. W. 2011. On the relation between loudness and the increased song frequency of urban birds. *Animal Behaviour*, 82, 831-836.

Cardoso, G. C., Mota, P. G. & Depraz, V. 2007. Female and male serins (Serinus serinus) respond differently to derived song traits. *Behavioral Ecology and Sociobiology*, 61, 1425-1436.

Catchpole, C. K. 1982. The Evolution of Bird Sounds in Relation to Mating and Spacing Behavior. In: *Acoustic Communication in Birds* (Ed. by D. E. Kroodsma & E. H. Miller), pp. 297-319. New York: Academic Press.

Catchpole, C. K. & Slater, P. J. B. 2008. *Bird Song: Biological Themes and Variations*. Cambridge: Cambridge University Press.

Clergeau, P., Croci, S., Jokimaki, J., Kaisanlahti-Jokimaki, M. L. & Dinetti, M. 2006. Avifauna homogenisation by urbanisation: Analysis at different European latitudes. *Biological Conservation*, 127, 336-344.

Clucas, B. & Marzluff, J. M. 2012. Attitudes and Actions Toward Birds in Urban Areas: Human Cultural Differences Influence Bird Behavior. *The Auk*, 129, 8-16.

Cody, M. L. & Brown, J. H. 1969. Song asynchrony in neighbouring bird species. *Nature*, 222, 778-&.

Collins, S. 2004. Vocal fighting and flirting: the functions of birdsong. In: *Nature's Music: The Science of Birdsong* (Ed. by P. Marler & H. Slabbekoorn), pp. 39-79. San Diego: Elsevier Academic Press.

Cramp, S. 1998. The Complete birds of the Western Paleartic In: *CD-ROM*: Oxford University Press & Optimedia.

Díaz, M., Parra, A. & Gallardo, C. 2011. Serins respond to anthropogenic noise by increasing vocal activity. *Behavioral Ecology*, 22, 332-336.

Dowling, J. L., Luther, D. A. & Marra, P. P. 2012. Comparative effects of urban development and anthropogenic noise on bird songs. *Behavioral Ecology*, 23, 201-209.

Foote, A. D., Osbornet, R. W. & Hoelzel, A. R. 2004. Whale-call response to masking boat noise. *Nature*, 428, 910.

Forrest, T. G. 1994. From Sender to Receiver: Propagation and Environmental Effects on Acoustic Signals. *American Zoologist*, 34, 644-654.

Francis, C. D., Ortega, C. P. & Cruz, A. 2011. Different behavioural responses to anthropogenic noise by two closely related passerine birds. *Biology Letters*, 7, 850-852.

Funghi, C., Cardoso, G. C. & Mota, P. G. 2013. Increased syllable rate is agressive in a bird with complex songs. *submitted to Animal Behaviour*.

Greenewalt, C. 1968. *Bird song: Acoustics and Physiology*. Washington: Smithsonian Institution.

Gross, K., Pasinelli, G. & Kunc, H. P. 2010. Behavioral Plasticity Allows Short-Term Adjustment to a Novel Environment. *The American Naturalist*, 176, 456-464

Halfwerk, W. & Slabbekoorn, H. 2009. A behavioural mechanism explaining noise-dependent frequency use in urban birdsong. *Animal Behaviour*, 78, 1301-1307.

Hamao, S., Watanabe, M. & Mori, Y. 2011. Urban noise and male density affect songs in the great tit Parus major. *Ethology Ecology & Evolution*, 23, 111-119.

Hartley, R. & Suthers, R. 1989. Airflow and pressure during canary song: direct evidence for mini-breaths. *Journal of Comparative Physiology A*, 165, 15-26.

Hauser, M. D. & Konishi, M. 1999. *The Design of Animal Communication*. London: MIT Press.

Hu, Y. & Cardoso, G. C. 2009. Are birds species that vocalize at higher frequencies preadapted to inhabit noisy urban areas? *Behavioral Ecology*, 20, 1268-1273.

Hu, Y. & Cardoso, G. C. 2010. Which birds adjust the frequency of vocalizations in urban noise? *Animal Behaviour*, 79, 863-867.

Klump, G. M. 1996. Bird communication in the noisy world. In: *Ecology and Evolution of Acoustic Communication in Birds* (Ed. by D. E. Kroodsma & E. H. miller), pp. 321-338. Ithaca: Cornell University Press.

Kroodsma, D. 2004. The diversity and plasticity of birdsong. In: *Nature's Music: The Science of Birdsong* (Ed. by P. Marler & H. Slabbekoorn), pp. 108-131. San Diego: Elsevier Academic Press.

Lengagne, T., Aubin, T., Lauga, J. & Jouventin, P. 1999. How do king penguins (Aptenodytes patagonicus) apply the mathematical theory of information to communicate in windy conditions? *Proceedings of the Royal Society B-Biological Sciences*, 266, 1623-1628.

Lengagne, T. & Slater, P. J. B. 2002. The effects of rain on acoustic communication: tawny owls have good reason for calling less in wet weather. *Proceedings of the Royal Society B-Biological Sciences*, 269, 2121-2125.

Luther, D. & Baptista, L. 2009. Urban noise and the cultural evolution of bird songs. *Proceedings of the Royal Society B-Biological Sciences*, 277, 469-473.

Luther, D. A. & Derryberry, E. P. 2012. Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. *Animal Behaviour*, 83, 1059-1066.

Mamede, A. T. & Mota, P. G. 2012. Limited Inter-Annual Song Variation in the Serin (Serinus serinus). *Ethology*, n/a-n/a.

Mamede, A. T. & Mota, P. G. unpublished. Effect of anthropogenic noise on serin song, Serinus Serinus.

McLaughlin, K. E. & Kunc, H. P. 2013. Experimentally increased noise levels change spatial and singing behaviour. *Biology Letters*, 9.

Mota, P. G. 1995. Ecologia Comportamental da Reprodução no Serino (*Serinus serinus*, Aves: Fringilidae), University of Coimbra.

Mota, P. G. 1999. The functions of song in the serin. *Ethology*, 105, 137-148.

Mota, P. G. & Cardoso, G. C. 2001. Song organisation and patterns of variation in the serin (*Serinus serinus*). *Acta Ethologica*, 3, 141-150.

Mota, P. G. & Depraz, V. 2004. A test of the effect of male song on female nesting behaviour in the serin (*Serinus serinus*): A field playback experiment. *Ethology*, 110, 841-850.

Nelson, D. A. 1988. Feature Weighting in Species Song Recognition By the Field Sparrow (Spizella Pusilla). *Behaviour*, 106, 158-181.

Nelson, D. A. 1989. Song frequency as a cue for recognition of species and individuals in the field sparrow (*Spizella pusilla*). *Journal of Comparative Psychology*, 103, 171-176.

Nemeth, E. & Brumm, H. 2009. Blackbirds sing higher-pitched songs in cities: adaptation to habitat acoustics or side-effect of urbanization? *Animal Behaviour*, 78, 637-641.

Parris, K. M. & Schneider, A. 2008. Impacts of traffic noise and traffic volume on birds of roadside habitats. *Ecology and Society*, 14.

Podos, J. 1997. A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes:Emberizidae). *Evolution*, 51, 537-551.

Proppe, D. S., Sturdy, C. B. & St. Clair, C. C. 2013. Anthropogenic noise decreases urban songbird diversity and may contribute to homogenization. *Global Change Biology*, 19, 1075-1084.

Quinn, J. L., Whittingham, M. J., Butler, S. J. & Cresswell, W. 2006. Noise, predation risk compensation and vigilance in the chaffinch *Fringilla coelebs*. *Journal of Avian Biology*, 37, 601-608.

Redondo, P., Barrantes, G. & Sandoval, L. 2013. Urban noise influences vocalization structure in the House Wren Troglodytes aedon. *Ibis*, 155, 621-625.

Reijnen, R. & Foppen, R. 1994. The effects of car traffic on breeding populations in woodland.1. Evidence of reduced habitat quality for willow warblers (*Phylloscopus trochilus*) breeding close to a highway. *Journal of Applied Ecology*, 31, 85-94.

Reijnen, R., Foppen, R., Terbraak, C. & Thissen, J. 1995. The effects of car traffic on breeding bird populations in woodland. 3. Reduction of density in relation to the proximity of main roads. *Journal of Applied Ecology*, 32, 187-202.

Rheindt, F. E. 2003. The impact of roads on birds: Does song frequency play a role in determining susceptibility to noise pollution? *Journal Fur Ornithologie*, 144, 295-306.

Ripmeester, E. A. P., Mulder, M. & Slabbekoorn, H. 2010. Habitat-dependent acoustic divergence affects playback response in urban and forest populations of the European blackbird. *Behavioral Ecology*, 21, 876-883.

Rogers, L. J. & Kaplan, G. 2002. *Songs, roars and rituals: Communication in birds, mammals and other animals*. Cambridge, Massachusetts: Harvard University Press.

Rowe, C. 1999. Receiver psychology and the evolution of multicomponent signals. *Animal Behaviour*, 58, 921-931.

Searcy, W. A. & Beecher, M. D. 2009. Song as an aggressive signal in songbirds. *Animal Behaviour*, 78, 1281-1292.

Shannon, C. E. & Weaver, W. 1948. The mathematical theory of communication (reprinted). *The Bell System Technical Journal*, 27, 379-423.

Slabbekoorn, H. 2004. Singing in the wild: the ecology of birdsong. In: *Nature's Music: The Science of Birdsong* (Ed. by P. Marler & H. Slabbekoorn), pp. 178-205. San Diego: Elsevier Academic Press.

Slabbekoorn, H. & den Boer-Visser, A. 2006. Cities Change the Songs of Birds. *Current Biology*, 16, 2326-2331.

Slabbekoorn, H. & Peet, M. 2003. Ecology: Birds sing at a higher pitch in urban noise. *Nature*, 424, 267.

Slabbekoorn, H. & Ripmeester, E. A. P. 2008. Birdsong and anthropogenic noise: implications and applications for conservation. *Molecular Ecology*, 17, 72-83.

Slabbekoorn, H. & Smith, T. B. 2002. Habitat-dependent song divergence in the little greenbul: An analysis of environmental selection pressures on acoustic signals. *Evolution*, 56, 1849-1858.

Slater, P. J. B. 1983. The study of communication. In: *Animal Behaviour* (Ed. by T. R. Halliday & P. J. B. Slater), pp. 9-42. Oxford: Blackwell Scientific Publications.

Slater, P. J. B. & Ince, S. A. 1979. Cultural evolution in chaffinch song. *Behaviour*, 71, 146-166.

Suthers, R. A. & Goller, F. 1997. Motor correlates of vocal diversity in songbirds. *Current Ornithology, Vol 14*, 14, 235-288.

Swaddle, J. P. & Page, L. C. 2007. High levels of environmental noise erode pair preferences in zebra finches: implications for noise pollution. *Animal Behaviour*, 74, 363-368.

Thorpe, W. H. 1958. The learning of song patterns by birds, with especial reference to the song of the chaffinch Fringilla coelebs. *Ibis*, 100, 535-570.

Tomecek, S. M. 2009. *Animal Behavior: Animal Communication*: Chelsea House.

Turner, W. R., Nakamura, T. & Dinetti, M. 2004. Global urbanization and the separation of humans from nature. *BioScience*, 54, 585-590.

United Nations. 2011. World Population Prospects: The 2010 Revision. (Ed. by P. D. Department of Economic and Social Affairs). New York.

United Nations. 2013. Worl population prospects: the 2012 revision. (Ed. by P. D. Department of Economic and Social Affairs). New York.

Verzijden, M. N., Ripmeester, E. A. P., Ohms, V. R., Snelderwaard, P. & Slabbekoorn, H. 2010. Immediate spectral flexibility in singing chiffchaffs during experimental exposure to highway noise. *Journal of Experimental Biology*, 213, 2575-2581.

Wallschläger, D. 1980. Correlation of song frequency and body weight in passerine birds. *Cellular and Molecular Life Sciences*, 36, 412-412.

Wiley, R. H. 1983. The evolution of communication: Information and manipulation. In: *Animal Behaviour* (Ed. by T. R. Halliday & P. J. B. Slater), pp. 156-189. Oxford: Blackwell Scientific Publications.

Wiley, R. H. & Richards, D. G. 1982. Adaptations for acoustic communication in birds: Sound transmission and signal detection. In: *Acoustic communication in Birds* (Ed. by D. E. Kroodsma & E. H. Miller), pp. 131-181. New York: Academic Press.

Wood, W. E., Yezerinac, S. M. & Dufty, J. A. M. 2006. Song parrow (*Melospiza melodia*) song varies with urban noise. *The Auk*, 123, 650-659.

Zollinger, S. A., Podos, J., Nemeth, E., Goller, F. & Brumm, H. 2012. On the relationship between, and measurement of, amplitude and frequency in birdsong. *Animal Behaviour*, 84, e1-e9.

Appendix

Appendix I – Material and Methods

(A) Study area and GPS coordinates

Local	GPS coordinates
City Park	40°12'3.50"N - 8°25'31.51"W
Jardim da Sereia	40°12'30.15"N - 8°25'3.23"W
Santa Clara	40°12'13.95"N - 8°26'5.24"W
Coimbra Higher School of Agriculture	40°12'36.66"N - 8°27'5.90"W
University of Coimbra Astronomical Observatory	40°11'58.16"N - 8°26'35.11"W
Hospital Centre of Coimbra	40°11'45.98"N - 8°27'40.31"W
Pereira	40°10'36.21"N - 8°35'5.09"W
Arzila	40°10'25.83"N - 8°34'3.42"W
Figueiró do Campo	40° 8'52.35"N - 8°34'16.96"W

Appendix II – Material and Methods

(A) Determination of song parameters for individuals from high-frequency group.

Subject	Stimulus	Phase	Song duration	Interval duration	Interval duration (log)	No. syllables trill	Peak freq song	Peak freq syllable	Section duration	Peak freq section	Environm. noise
1	High	Before	2,863	5,475	0,738	8	6612	8697	0,724	6345	
		During	3,270	2,374	0,375	12	6848	8760	0,645	6675	46,5
		After	4,997	2,821	0,450	19	6444	8603	0,736	5830	
2	High	Before	4,523	2,607	0,416	16	7166	8467	1,102	6530	
		During	3,683	3,518	0,546	7	6642	8557	1,109	6230	44,0
		After	4,112	5,035	0,702	11	6274	8540	1,099	6560	
3	High	Before	4,202	1,076	0,032	8	6154	9600	1,196	6013	
		During	4,298	3,743	0,573	8	6136	9815	1,205	6620	46,5
		After	3,378	1,943	0,288	8	6662	9675	1,212	6043	
4	High	Before	2,214	6,576	0,818	1	6296	8348	0,670	7196	
		During	4,717	8,007	0,903	2	6736	8434	0,691	7184	47,8
		After	3,261	19,575	1,292	0	6278	8338	0,684	7254	
5	High	Before	1,450	2,934	0,467	6	5762	8245	0,345	6420	
		During	3,325	2,926	0,466	11	7062	8270	0,342	6650	51,3
		After	3,666	3,986	0,601	22	6184	8315	0,352	6960	
6	High	Before	4,229	1,682	0,226	16	6128	8948	0,525	5900	
		During	4,705	1,857	0,269	13	6276	9022	0,485	5693	49,9
		After	3,035	2,705	0,432	9	6194	8900	0,522	6020	
7	High	Before	4,768	4,523	0,655	35	6586	8340	0,791	5855	
		During	5,166	2,965	0,472	20	5979	8263	0,787	5785	49,9
		After	6,389	2,587	0,413	27	5925	8323	0,802	6490	

(A) (continuation) Determination of song parameters for individuals from high-frequency group.

Subject	Stimulus	Phase	Song duration	Interval duration	Interval duration (log)	No. syllables trill	Peak freq song	Peak freq syllable	Section duration	Peak freq section	Environm. noise
8	High	Before	1,198	6,266	0,797	14	5958	8387	-	-	
		During	1,217	6,473	0,811	14	6324	8433	-	-	52,4
		After	1,106	7,880	0,897	13	6073	8260	-	-	
9	High	Before	3,592	3,811	0,581	19	6414	7965	0,965	6090	
		During	4,337	7,930	0,899	27	6380	7990	0,958	6020	46,5
		After	2,522	3,589	0,555	21	6218	8030	0,949	6065	
10	High	Before	3,308	2,039	0,309	8	6100	8830	0,989	5830	
		During	3,810	2,942	0,469	8	5940	8975	0,983	5900	43,4
		After	2,660	3,000	0,477	3	5862	7075	0,990	5875	
11	High	Before	2,920	1,869	0,272	5	6772	8713	0,922	6357	
		During	2,462	2,573	0,410	6	6426	8790	0,915	6340	44,8
		After	2,798	3,412	0,533	4	6260	8743	0,909	6260	
12	High	Before	3,705	2,448	0,389	10	6044	7213	0,958	6403	
		During	3,485	4,729	0,675	18	5986	9903	0,969	5980	47,5
		After	3,307	4,039	0,606	8	6062	10057	0,953	5980	
13	High	Before	6,763	6,264	0,797	35	6024	8590	1,087	7573	
		During	5,717	3,648	0,562	26	6174	8827	1,081	6107	52,6
		After	4,428	5,091	0,707	30	6166	8620	1,092	6447	
14	High	Before	3,293	1,891	0,277	25	6492	8340	1,269	5844	
		During	2,962	2,830	0,452	15	6182	8452	1,244	5780	43,3
		After	3,859	2,166	0,336	22	6304	8328	1,259	5836	
15	High	Before	5,228	2,538	0,405	12	5986	8715	0,693	6340	
		During	5,420	3,025	0,481	15	6354	8715	0,707	6337	48,5
		After	4,095	3,682	0,566	10	6070	8760	0,708	6417	

(B) Determination of song parameters for individuals from low-frequency group.

Subject	Stimulus	Phase	Song duration	Interval duration	Interval duration (log)	No. syllables trill	Peak freq song	Peak freq syllable	Section duration	Peak freq section	Enviromm. noise
16	Low	Before	4,280	1,156	0,063	11	6522	4387	1,152	6698	4387
		During	4,446	3,084	0,489	2	6266	4197	1,131	6088	4197
		After	4,112	1,787	0,252	2	6612	4293	1,132	5925	4293
17	Low	Before	1,351	8,201	0,914	0	7740	4420	-	-	4420
		During	1,381	7,196	0,857	0	7680	4374	-	-	4374
		After	1,656	5,943	0,774	0	7720	4350	-	-	4350
18	Low	Before	6,005	3,251	0,512	31	5855	4660	0,941	5645	4660
		During	4,299	3,155	0,499	16	6062	4800	0,934	5690	4800
		After	5,488	3,406	0,532	17	5732	4685	0,921	5765	4685
19	Low	Before	1,376	5,201	0,716	14	7186	3508	-	-	3508
		During	1,818	5,114	0,709	16	6970	3398	-	-	3398
		After	2,084	4,823	0,683	17	6220	3672	-	-	3672
20	Low	Before	4,894	7,733	0,888	11	6772	3510	1,010	6615	3510
		During	5,218	3,813	0,581	12	6312	3510	1,012	6268	3510
		After	4,198	3,222	0,508	9	6116	3650	1,010	5928	3650
21	Low	Before	3,321	3,238	0,510	12	6464	2830	-	-	2830
		During	2,699	3,099	0,491	9	6124	2880	-	-	2880
		After	4,316	2,928	0,467	21	6116	2760	-	-	2760
22	Low	Before	4,148	4,633	0,666	16	6352	3633	-	-	3633
		During	4,290	3,761	0,575	17	6932	3587	-	-	3587
		After	2,622	3,571	0,553	3	7158	3607	-	-	3607

(B) (continuation) Determination of song parameters for individuals from low-frequency group.

Subject	Stimulus	Phase	Song duration	Interval duration	Interval duration (log)	No. syllables trill	Peak freq song	Peak freq syllable	Section duration	Peak freq section	Environm. noise
23	Low	Before	4,657	3,448	0,538	22	6248	3807	1,082	5213	
		During	4,229	3,353	0,525	26	6164	3760	1,082	5230	46,0
		After	3,176	2,568	0,410	19	5978	3733	1,095	5290	
24	Low	Before	1,247	5,327	0,726	2	7128	4120	-	-	
		During	1,265	5,428	0,735	3	7006	4005	-	-	45,0
		After	1,240	5,193	0,715	3	6266	4097	-	-	
25	Low	Before	3,336	1,573	0,197	10	6174	3784	1,067	6238	
		During	3,265	3,161	0,500	9	5950	3680	1,072	6092	46,7
		After	2,427	3,012	0,479	6	6014	3784	1,067	6304	
26	Low	Before	4,182	5,498	0,740	31	6372	4295	-	-	
		During	4,745	2,083	0,319	31	6596	4343	-	-	43,5
		After	6,737	5,657	0,753	47	6490	4225	-	-	
27	Low	Before	3,146	1,536	0,186	8	6154	-	-	-	
		During	2,316	2,725	0,435	8	6316	-	-	-	47,0
		After	2,706	1,812	0,258	8	6700	-	-	-	
28	Low	Before	2,993	2,912	0,464	5	5778	3405	-	-	
		During	3,276	5,269	0,722	7	5948	3348	-	-	47,8
		After	3,013	4,912	0,691	6	5910	3315	-	-	
29	Low	Before	4,552	3,123	0,495	13	6762	4667	0,960	6527	
		During	3,785	2,948	0,470	17	6952	4840	0,936	6573	60,0
		After	3,432	3,040	0,483	18	6764	4493	0,943	6217	
30	Low	Before	3,317	2,407	0,381	12	6662	4680	-	-	
		During	3,180	1,762	0,246	17	6520	4540	-	-	49,5
		After	5,302	8,732	0,941	12	6990	4500	-	-	

(B) (continuation) Determination of song parameters for individuals from low-frequency group.

Subject	Stimulus	Phase	Song duration	Interval duration	Interval duration (log)	No. syllables trill	Peak freq song	Peak freq syllable	Section duration	Peak freq section	Environm. noise
31	Low	Before	3,283	5,470	0,738	15	6239	3670	0,900	6294	
		During	3,160	5,411	0,733	18	6044	3763	0,901	6060	51,2
		After	2,143	7,243	0,860	14	6192	3480	0,881	6026	
32	Low	Before	4,271	4,444	0,648	10	6942	4190	1,172	7075	
		During	3,573	3,356	0,526	7	7016	4240	1,153	6720	54,5
		After	3,823	5,607	0,749	15	7120	4330	1,153	7120	
33	Low	Before	5,331	3,175	0,502	26	6026	4230	-	-	
		During	5,676	5,181	0,714	29	6043	4447	-	-	48,2
		After	6,646	14,730	1,168	27	6248	4353	-	-	
34	Low	Before	4,546	5,183	0,715	9	6220	3243	1,286	6580	
		During	5,264	2,494	0,397	18	6698	3255	1,300	6485	48,5
		After	4,690	7,021	0,846	24	6744	3298	1,270	6510	

Appendix III – Material and Methods

(A) Number of song sung by individuals from high-frequency group. Individuals 7 and 8 sang only 4 and 3 songs, respectively, in phase after stimulus (in grey).

Subject	Date	Stimulus	No. Songs before stm	No. Songs during stm	No. Songs after stm
1	16-04-2013	High	5	5	5
2	16-04-2013	High	5	5	5
3	18-04-2013	High	5	5	5
4	18-04-2013	High	5	5	5
5	19-04-2013	High	5	5	5
6	19-04-2013	High	5	5	5
7	19-04-2013	High	5	5	4
8	19-04-2013	High	5	5	3
9	19-04-2013	High	5	5	5
10	25-04-2013	High	5	5	5
11	16-05-2013	High	5	5	3
12	21-05-2013	High	5	5	5
13	22-05-2013	High	5	5	5
14	23-05-2013	High	5	5	5
15	28-05-2013	High	5	5	5

(B) Number of song sung by individuals from low-frequency group. Individual number 18 sang only 4 songs in phase before stimulus (in grey).

Subject	Date	Stimulus	No. songs before stm	No. Songs during stm	No. Songs after stm
16	17-04-2013	Low	5	5	5
17	17-04-2013	Low	5	5	5
18	27-04-2013	Low	4	5	5
19	27-04-2013	Low	5	5	5
20	01-05-2013	Low	5	5	5
21	01-05-2013	Low	5	5	5
22	10-05-2013	Low	5	5	5
23	12-05-2013	Low	5	5	5
24	13-05-2013	Low	5	5	5
25	13-05-2013	Low	5	5	5
26	14-05-2013	Low	5	5	5
27	14-05-2013	Low	5	5	5
28	15-05-2013	Low	5	5	5
29	16-05-2013	Low	5	5	5
30	16-05-2013	Low	5	5	5
31	21-05-2013	Low	5	5	5
32	22-05-2013	Low	5	5	5
33	27-05-2013	Low	5	5	5
34	27-05-2013	Low	5	5	5